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14. ABSTRACT:

This project will lead to the first biodegradable "green" corneal bandage that accelerates corneal healing. The bandage resembles a contact lens. When this bandage is placed on a wounded eye it reduces inflammation and stimulates the healing process. It is made of a novel biomaterial, which can be programmed to "dissolve" within hours to days providing patients with a tailored product. The broader impact/commercial potential of this project will help the 2 million Americans that sustain traumatic injuries to the cornea each year, and the 4 million Americans that undergo surgery annually leaving the cornea wounded. Such corneal wounds cause intense pain and may lead to blindness depending on the severity. This new eye bandage accelerates corneal healing and adheres to the surface of the eye to aid in alleviating pain. Production is fully scalable to large quantities, and can be easily packaged and distributed in a similar fashion as a contact lens. Furthermore, the eye bandage is an innovative technology, patented, and new to the medical device industry.

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Introduction

Over 6 million Americans sustain traumatic injuries to the cornea each year from traumatic incidents and surgical procedures (*1*, *2*). Such corneal wounds cause intense pain and may lead to blindness depending on the severity of injury. Sarentis Ophthalmics, Inc., in collaboration with the Weill Cornell Medical College is developing the first biodegradable "green" bandage that accelerates corneal healing. The bandage resembles a contact lens and is produced from silk derived proteins, which have been shown to be highly biocompatible and non-immunogenic when implanted within the body (*3*-*5*). When this bandage is placed on a wounded eye it is designed to reduce inflammation and stimulate the healing process (*6*). It is made of a novel biomaterial, which can be programmed to "dissolve" within hours to days and provide patients with a tailored product optimized for their traumatic condition. Sarentis' corneal bandage accelerates corneal healing and self-adheres to the surface of the eye to aid in alleviating pain. This regenerative bandage is inexpensive and is sterilely produced and packaged for less than a few pennies. The use of this product is expected to lessen procedure expense for treating cornea trauma resulting in millions in savings to the American health care system, while allowing for the expansion of eye injury in the point of care environment (i.e. hostile setting, clinic, home use, etc.) (*7*, *8*).

Body

During the project's first year, Sarentis Ophthalmics was able to demonstrate feasibility that the silk bandage can be produced and used to help expedite corneal wound healing. *In vivo* results indicate that the residence time of the silk bandage upon the wound site correlates to corneal healing rate, in that too minimal of a residence time has no effect on healing, while too long of a residence time slows down healing. Animal testing indicates that optimized dissolution time is estimated to between 8-12 hours to stimulate maximum healing efficacy. Therefore, greater understanding into how to control the material's dissolution upon the surface of the eye is required. Work throughout the second year of the award has focused on gaining a better understanding of how to control these material dissolution properties through use of the water-annealing (WA) process (*9*, *10*). Initial results indicate that WA will effectively control silk bandage solubility with desirable working range applicable for the clinical development (*10*). In addition, the company has also focused on translating these laboratory findings

into a manufactured product through implementing production practices within a good manufacturing practice (GMP) environment under an ISO quality system in preparation for FDA submission. The culmination of these previous efforts in combination with future development plans, it is anticipated that first-in-man will be accomplished by October 2013.

Technical Progress

Task 1. Irritation response

This portion of the project was completed during the first year of work and therefore there are no updates to report at this time.

Task 2. Burn wound assessment

This portion of the project was completed during the first year of work and therefore there are no updates to report at this time.

Task 3. Abrasive wound assessment

This portion of the project was completed during the first year of work and therefore there are no updates to report at this time.

Task 4. Puncture wound assessment

Intrastromal Corneal Toxicity

Before moving forward on puncture wound closure assessment, studies were conducted to assess the potential toxicity that the silk fibroin protein may have on corneal tissue. This is especially a concern with relation to puncture wounds, as there is the potential that non-dissolved silk protein may reside in the wound site for extended periods of time and produce undesired consequences. To explore this potential toxicity effect, the silk material was placed intrastromally within the rabbit cornea model. To do this, silk fibroin films that measured 6-mm in diameter and 3-μms in thickness were cast and then WA for 4-hours to produce an insoluble film sample representative of non-dissolved bandage material (*11*). The silk film physical dimensions were chosen to maximize the amount of implanted material by minimizing potential effects that could stem from the reduction of oxygen and nutrients to the cornea by keeping the material thin while covering the majority of the cornea area (*12*). The silk films were implanted

into the anterior segment of the rabbit cornea by producing a tissue flap of 8-mm diameter and 150-μm in depth with a surgical blade. The film was then placed on the stroma bed, and then covered by the flap [Figure 1].

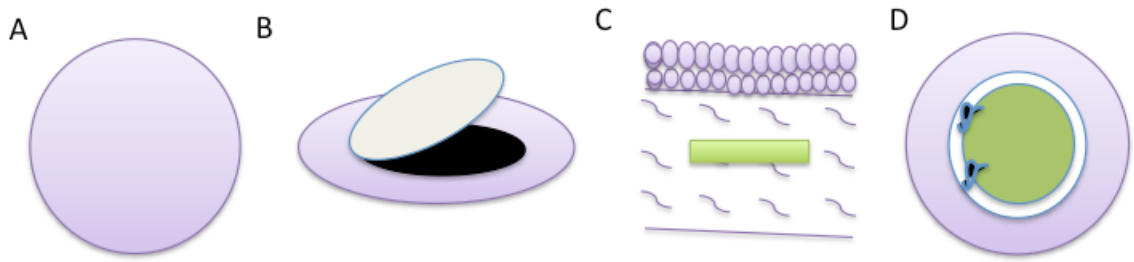


Figure 1. (A) The rabbit corneal anterior segment was partially excised by creating a (B) tissue flap. (C) The silk film was then placed within the stroma matrix, and (D) the flap was sutured closed.

Postoperative ocular inflammation, neutrophil infiltration, corneal wound healing, neovascularization, infection and integrity of the silk film were examined using a slit lamp microscope [Figure 2A-D]. Corneal reepithelialization was completed within 1 week post-op with no apparent inflammatory response observed [Figure 2A]. The cornea remained transparent and free from inflammation at both 4 [Figure 2B] and 12 weeks [Figure 2C] post-surgery, which compared favorably to controls [Figure 2D]. *In vivo* corneal architecture was examined by optical coherence tomography (OCT) at 12 weeks post-surgery, which revealed normal tissue structure and absence of inflammation [Figure 2E]. Rabbits with and without silk implants were sacrificed at 1 and 3 months respectively. H&E staining of corneal histology sections was performed to examine the corneal structure, inflammatory response, and silk film degradation [Figure 2F-H]. The silk film remained transparent and still present in the cornea tissue 4 weeks post-surgery [Figure 2F]. The silk films appeared to integrate and degrade within the stroma matrix after 3 months with no sign of inflammation [Figure 2G], which compared favorably to controls [Figure 2H]. These results indicate that the silk bandage material does not produce a toxicity response upon long-term exposure to the cornea tissue. Furthermore, it is reasonable to assume that embedment of non-dissolved bandage particulates within a puncture wound injury will not adversely affect corneal tissue homeostasis. Further work will be undertaken to assess the effectiveness of

puncture wound closure using the silk bandage product.

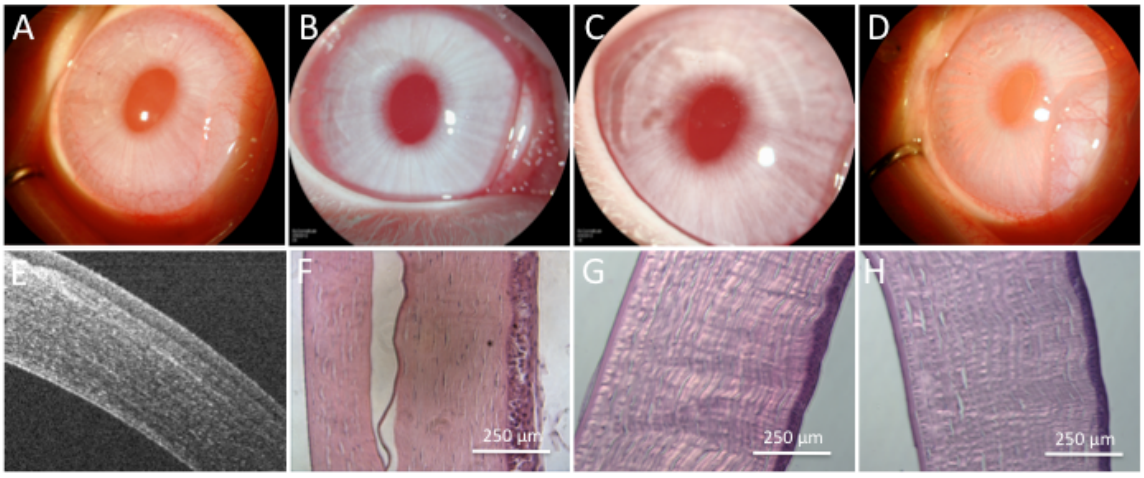


Figure 2. Intrastromal silk film implants in the rabbit cornea. Slit lamp photographs at (A) 1, (B) 6, and (C) 12 weeks post-surgery, and (D) control at 12 weeks. (E) OCT image of cornea at 12 weeks post-op demonstrating absence of inflammatory cells. H&E staining of cornea cross-sections at (F) 6 and (G) 12 weeks post implantation, and (H) control at 12 weeks.

Task 5. GMP production of a regenerative lens

Heat-annealing (HA) Process: *In Vitro* Assessment

During the course of year 1 the silk bandage prototype was successfully produced on the laboratory bench. However, more work is required to optimize the material dissolution rate to maximize corneal surface regeneration potential. Previously the established water-annealing (WA) method was utilized to modify dissolution potential, but this method proved difficult to control and would require a more sophisticated approach to finely control environmental parameters (*10*). Therefore, an alternative heat annealing (HA) process was employed as an attempt to control dissolution of the silk bandage while offering the potential advantage of simultaneous sterilization. In brief, HA involves placing the silk bandage into a dry oven for a given period of time. The temperature of the oven is typically set between 140°C-170°C, and the material is heated for 1.5-2.5 hours (i.e. 30 minute increase per 10°C decrease) as mandated by FDA sterilization guidelines.

To better aid in studying silk film dissolution, an *in vitro* bench test was optimized using a slight modification of the Bradford protein assay. This assay enabled faster

assessment of silk film dissolution without having to subject animals to material testing. This enhanced understanding into how silk material processing parameters affect the extent of material dissolution. Silk film samples were heated at different temperatures for varying time periods, and results indicated that this processing method was found to reduce silk material dissolution with increasing temperature and heating time (Figure 3A). There was a 40% decrease in material dissolution between controls and samples heated at 180°C for 120 minutes. These results indicate that heat may be used to modify material dissolution characteristics.

To better understand the mechanism for the decrease in material dissolution, silk sample water content and secondary structure were analyzed using thermal gravimetric analysis (TGA) and Fourier-transform infrared (FTIR) spectroscopy respectively (*13*). TGA was performed on silk samples that were HA at 180°C to determine how water loss impacted decreasing material dissolution (*12*). Results indicated that the silk film water content decreased with heating time (Figure 3B), which correlates with a decrease in material dissolution. These observations indicate that the presence of water within the silk material is important for increased material dissolution. To better understand the effects of heating on protein secondary structure (i.e. beta-sheet and alpha helix content) the silk film samples were analyzed using FTIR spectroscopy. Results indicated that there was little change in the amide-I region of the spectrum compared to controls (Figure 3C), which indicates that the heating process was not affecting protein secondary structure conformation (*14*). These observations indicate that the HA process induces changes in material dissolution primarily through dehydrating the material and does not induce protein chain movement to form more thermodynamically stable secondary structures. This dehydration may indicate why silk dissolution rate decreases at high heating temperatures where water has been largely removed from the material, while retaining a high degree of dissolution at cooler heating regimes. In addition, it appears that dry heat will also suffice as an effective sterilization method, as it does not appear to affect the protein structure as indicated by FTIR analysis.

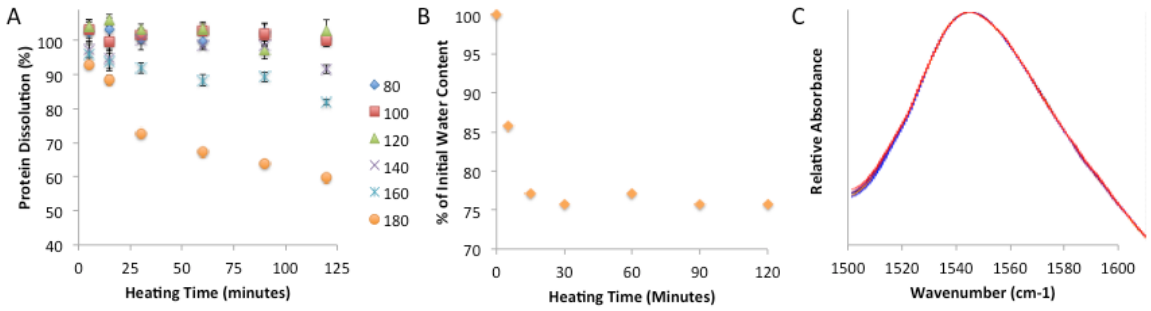


Figure 3. (A) Silk material dissolution profiles for samples heated at different temperatures and lengths of time (n = 4, error bars = SD). (B) Percent of initial water content held by the silk film samples heated at 180°C for 120 minutes (n = 4). Overlaid FTIR spectrum for silk films heated at various temperatures (RT, 140°C, 160°C, and 180°C) for up to 120 minutes.

Cryogenic Storage of Silk Solution

Storage of the raw silk solution is also important. Currently, silk solution has a shelf life of 2 months when stored at 4°C (*15*). Although 2 months is sufficient on the laboratory scale, this time frame would need to be extended to enable long-term inventory storage and offer more flexibility in future product development. Therefore, the dissolution of silk bandages composed of fresh silk solution frozen at -80°C and left at room temperature (RT) were compared to determine if cryogenic freezing would significantly effect silk bandage dissolution characteristics. Silk films 14-mm in diameter and 50-μm thick were cast from 8% silk solution that was thawed from -80°C and RT conditions (*11*). Silk films were then heated at various sterilizing temperatures (140°C -180°C) for varying periods of time (5, 15, 30, 60, 90, and 120 minutes).

The extent of silk film dissolution in water was then assessed using the modified Bradford protein assay as mentioned earlier. Results indicated that at the majority of heating temperatures and times did not significantly change silk film dissolution [Figure 4]. The only significant ($n = 4$, $p<0.05$) change in dissolution was for the 90 and 120 minutes time points for the highest 180°C heating condition. These results indicate that storing of silk solution at cryogenic conditions will not significantly alter silk material dissolution for the majority of conditions and may serve as a potential storage method for solution inventory. Further work will be needed to more fully characterize the effect of freezing on silk bandage material characteristics.

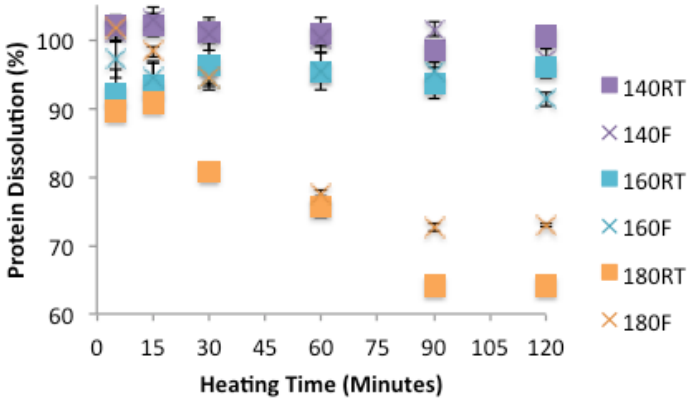


Figure 4. Silk film dissolution for samples made from solution stored at either RT or -80°C conditions. Samples were processed at various sterilizing temperatures between 140°C to 160°C and sampled at various times over a two hour period (n = 4, error bars = standard deviations).

Silk Bandage Adhesion and Dissolution Profile at the Cornea Surface

Further work in Q6 was undertaken to better understand silk bandage interaction with the cornea surface. Previously it was unknown if the material adhered to the surface or was floating on the tear film post application. In addition, the silk bandage dissolution profile was unknown, and it was unclear how the surrounding tear film fluid would affect the bandage swelling and dissolution properties. To better understand how the silk bandage interacts with the corneal surface, time-lapse OCT images were taken of silk bandages on a live rabbit model [Figure 5A]. Previous data from dissolution experiments on HA silk samples indicated that bandages heated at 150°C for 2 hours were soluble and would adhere to the corneal surface of a rabbit animal model, so this processing regime was utilized for OCT imaging. Upon initial application of the processed bandages the material adhered to the corneal surface and began to immediately swell in thickness. Wave morphologies were observed with material cross-sectional thickness ranging from 79 to 114-μm [Figure 5B]. After 1 minute post-application the silk bandage thickness had flattened out to around 100-μm, which corresponded to a 25% increase in bandage thickness [Figure 5C]. Over a 3-minute time period the thickness increased up to 136-μm, which corresponded to a 70% increase [Figure 5D-E]. The thickness then began to decrease as the material started to

dissolve after 4-minutes post application [Figure 5F]. After 10-minutes post-application the silk bandage thickness had reduced to around 100-μm in thickness, and the edge regions appeared to maintain both consistent thickness and attachment to the corneal surface [Figure 5G]. After a total of 45-minutes upon the eye portions non-dissolved particulates were observed on the cornea surface in various locations [Figure 5H]. The rabbit cornea appeared unaffected by the presence of the material, and the animal showed no signs of discomfort or subsequent inflammation after the silk bandage's application.

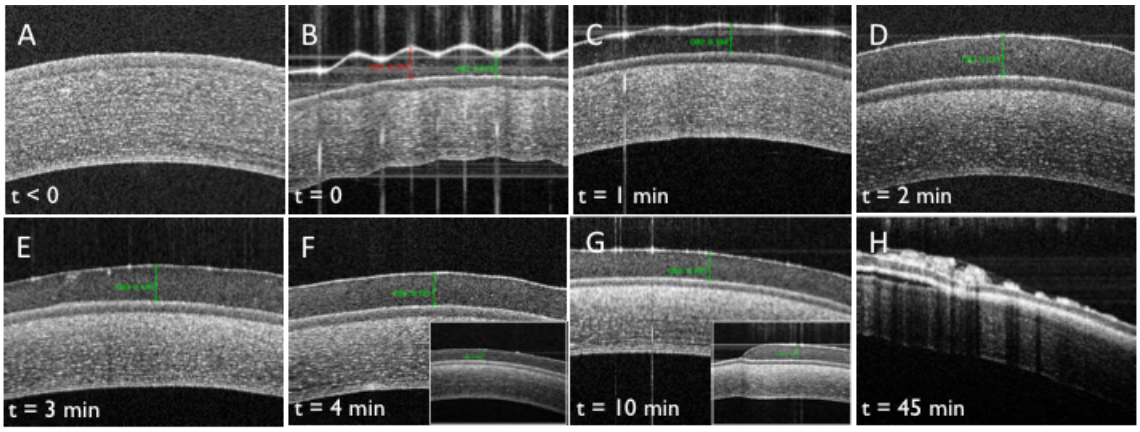


Figure 5. The silk bandage's initial adhesion to the (A) cornea was evaluated over time with OCT imaging from (B-F) 0 to 4-minutes, (G) 10-minutes, and (H) 45-minutes respectively. (B) The bandage was found to form wave morphologies upon initial adhesion, (C-E) then swelled as the material hydrated. (F-G) This was followed by a period of material dissolution as the bandage reduced in thickness. (G) The bandage edge showed uniform thickness and remained well adhered to the corneal surface after 10-minutes post application. (H) Insoluble portions of the silk bandage were measured at 45-minutes showing the remaining silk particulates.

These results indicate that the silk bandage is attaching to the cornea surface and absorbing fluid upon application. The material then hydrates and swells into a gel-like state, which then uniformly coats the surface and begins to dissolve with time. The bulk of the HA silk bandages appeared to dissolve within 45-minutes post-application. Comparatively, silk bandages that were not HA appear to dissolve within 5 minutes of application to the eye. Therefore, the HA bandage did appear to maintain a higher residence time when compared to untreated samples. However, it would be more

desirable to have a corneal residence time of several hours, as this appears to help stimulate wound healing. In summary, although HA bandages did possess substantially longer residence times than untreated bandages, this material processing technique is not optimal for the silk corneal bandage product.

Initial Characterization of Water-annealed Silk Film Dissolution

The limited residence time of the silk bandages achieved by utilizing the HA process stimulated further investigation into the WA process. An emptied glass desiccator with water in the basin was used as a WA chamber (*16*), and the environment was monitored with a relative humidity (RH) and temperature sensor. Silk film samples measuring 14-mm in diameter and 50-μm in thickness were cast using the same batch of silk solution as the bandages from the previous residence time studies. Initial studies demonstrated that silk film samples were successfully created that dissolved to varying degrees in water as a result of varying the WA processing time at 95% RH and 24°C [Figure 6A-D]. Unprocessed silk films had near complete dissolution in water [Figure 6A], while there was reduced dissolution for samples processed for 20-minutes where non-dissolved portions of the film remained translucent [Figure 6B]. It was shown that the edge of the silk films tended to dissolve to less of a degree than the central regions. Silk films that were processed for 40-minutes and above appeared to show limited dissolution in water and maintained transparency [Figure 6C-D].

Protein assay results using the previously described modified Bradford assay confirmed qualitative results indicating that protein dissolution was reduced with increased WA processing time after a 15 minutes incubation time in water [Figure 6E]. After 20 minutes there was a near 30% drop, which was much more rapid than what was observed with the HA process. However, the material dissolution appeared more uniform in nature, and complete insoluble materials were produced which was not the case in the HA process. In summary, these results indicate that there is a larger window of processing available with the WA method when compared to HA, however more work is required to understand how to better control the WA process.

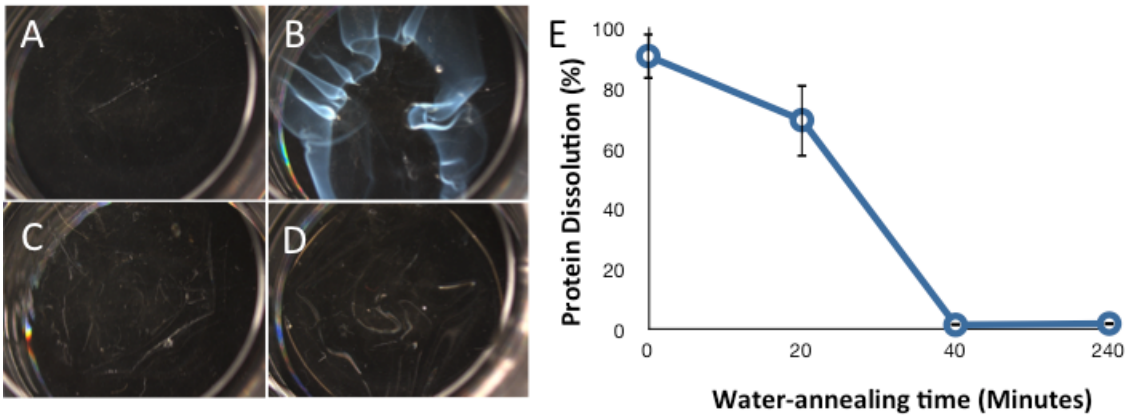


Figure 6. (A-D) Silk film dissolution in water was reduced with increasing WA processing time at (A) 0, (B) 20, (C) 40, and (D) 240-mintues. (A) Unprocessed control samples appeared to completely dissolve, (B) 20-minute processing time demonstrated partial dissolution, and (C-D) 40-minutes and above appeared to produce insoluble samples. (E) Bradford protein assay results confirmed qualitative assessment that the extent of silk sample dissolution in water reduced with WA processing time (n = 3, error bars = SD).

These results indicate that the primary variables in the WA process are temperature, relative humidity (RH), and processing time. The WA chamber temperature was modified by placing the entire apparatus in a ambient, heated (i.e. oven), or cooled (i.e. refrigerator) temperature condition. Silk film samples were treated at various temperatures, RH, and processing times, and then dissolved in water and assayed using the modified Bradford protein dissolution assay as previously described. Results indicate that when silk film samples are WA in a high RH environment (i.e. >90%) changes in chamber temperature and processing time drive changes in the silk material dissolution profile (Figure 7A). Specifically, the silk bandage dissolution decreases with increasing chamber temperature and processing time at high RH. Similarly, low temperature processing shows negligible changes in silk film dissolution even at high RH and for long periods of processing time (Figure 7B). These results indicate that the WA process can be successfully controlled and used to modify silk dissolution characteristics through changing the environmental temperature, RH, and processing time. These represent significant findings for the project, and indicate enhanced understanding into this important process for the silk bandage.

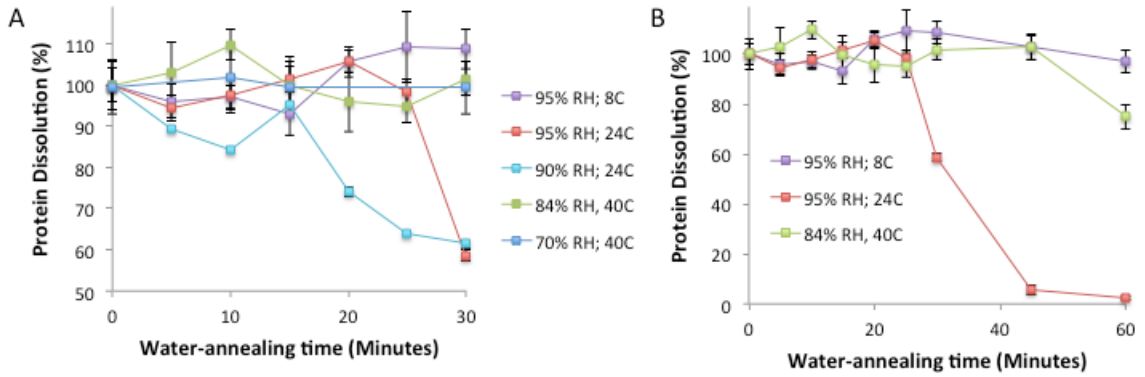
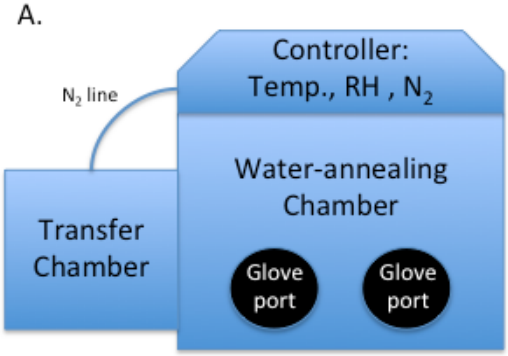


Figure 7. Protein dissolution versus WA processing time for samples processed at different RH (%) and temperature (°C) conditions. The extent of silk film dissolution can be controlled based on 3 factors: RH, temperature, and processing time. At high RH the extent of silk bandage dissolution can be modified in based on the environmental temperature, while decreasing RH increases temperature requirements. Total extent of material dissolution can then be optimized based on total processing time (n = 4, error bars = SD).

To aid in eliminating potential variability produced from the WA environment a more sophisticated environmental glove box chamber was designed and fabricated [Figure 8A]. The chamber controls temperature within 0.3°C and relative humidity within 2% variation [Figure 8B]. The additional environmental control allows for a predictable and reproducible processing regime, and has ample space for production [Figure 8C]. In addition, an N_2 drying transfer chamber will be attached to the processing chamber. This will allow for the complete drying of the silk film before and after the WA processing time. This will ensure greater consistency of silk bandage processing regardless of the outside environment (*17*). In addition, this will also reduce variations within the processing environment that can result from opening chamber doors. Finally, the chamber is equipped with sealed glove ports that will allow handling of materials within the processing chamber without risk of compromising the internal environment. Taken together, this apparatus will successfully control temperature, RH, and processing time to a much greater extent then the current laboratory setup.

A.



B.

Environmental Control Specifications			
Temperature		**Humidity**	
Temperature Range:	16-41° C	Humidity Range:	1-95% Rh
Temperature Sensor:	±0.3° C	Humidity Sensor:	±2% Rh
Resolution:	±1°	Control Accuracy:	±1% Rh
On Screen Datalogging:	0.1° C	Resolution:	0.1% Rh

C.

Physical Dimensions	
I.D.	28"w x 23"d x 29"h
O.D.	43"w x 30"d x 43"h
Top Opening	17" x 23"
Door Opening	15"w x 18"h
Chamber Vol.	11.2 cu. ft.
Weight	250 lbs.

Figure 8. (A) Schematic of the WA processing apparatus design consisting of a transfer chamber, WA chamber, and controller. (B) Specifications table listing important environmental control features of the design. (C) Listing of physical dimensions of the design.

Utilizing the environmental chamber, initial experiments were performed that processed the silk films at varying temperature (16, 17, 18, and 19°C) at dew point saturation (100% RH). Samples were then processed for 0, 10, 20, 30, 45 and 60 minutes within the environmental chamber and then removed for analysis. Results confirmed earlier experimental observations that by changing either temperature or processing time the silk fibroin material secondary structure and dissolution within water can be modulated. FTIR analysis revealed a change in silk fibroin protein secondary structure confirmation with a more prevalent beta-sheet peak formation at 1624 cm^{-1} with increasing processing temperature and after 60 minutes of processing time (Figure 9A). Unprocessed control samples do not contain this beta-sheet peak, however by increasing temperature this peak becomes more prevalent post processing. The formation of additional beta-sheets as observed in the FTIR spectrum corresponds to changes in silk bandage material dissolution (Figure 9B). Silk bandage material samples were also analyzed using the previously described water dissolution study. However, this experiment utilized an additional piece of instrumentation that detects protein concentration in solution through FTIR spectral analysis (Direct Detect device, EMD-Millipore), which provides a direct analysis of protein content and does not require indirect chemical analysis used in the Bradford assay. Results from this assay indicate that lower temperature processing at 16°C tended to preserve high solubility of the material within water even after 1 hour of processing time, similar to unprocessed

controls (i.e. 0 minutes). However, increasing the processing temperature to 17°C resulted in a significant decreases in material solubility that was also noted for 18°C and 19°C processing temperatures. These results together agree with previous results, which demonstrate that material dissolution in water may be altered by changing either processing temperature or time. With the added control over the WA process that is afforded by the new environmental chamber it is anticipated that silk dissolution time upon the cornea surface will be readily controlled as previously anticipated.

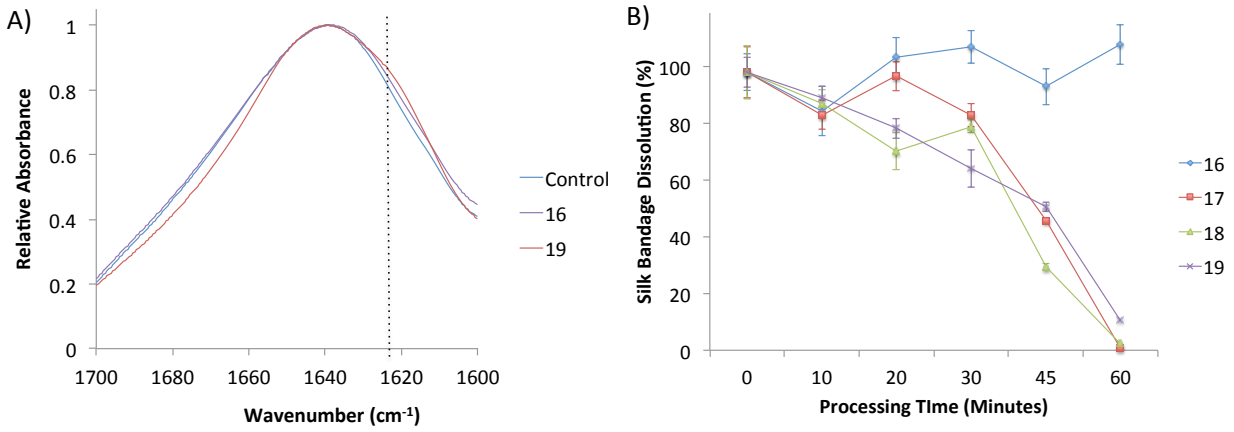


Figure 9. (A) An increase in the major silk protein beta-sheet spectral peak is observed at 1624 cm^{-1}. (B) Corresponding changes in total silk bandage sample dissolution in water with changing processing temperature and time was observed with both increased processing temperature (°C) and time.

Silk Solution Scale-Up, E-beam Sterilization Studies, and Packaging Development

During the final quarter of 2012 significant progress was made towards translating silk bandage development from the research bench to manufacturing production. Intense focus has been applied on translating the previous lab protocols to a GMP standard process. Efforts are underway to produce a standardized and automated silk solution production method for use in the clean room environment. Current lab protocols introduce non-satisfactory levels of variability into the production process (*16*). The goal of the new methodology will be to minimize direct user interaction, while producing a consistent product that will be straightforward to validate and reproduce. Two separate systems have been designed and are currently being implemented within the clean room setting. The first setup (Figure 10A) will be utilized to produce extracted silk fibroin fibers from raw silk worm cocoons, and the second (Figure 10B) will allow for automated

dialysis to produce the 8% wt./vol. base silk solution to produce the corneal bandage. When fully implemented the controllers for both units will allow for push button start to finish processing while supplying a consistent batch-to-batch production with real-time monitoring of silk production.

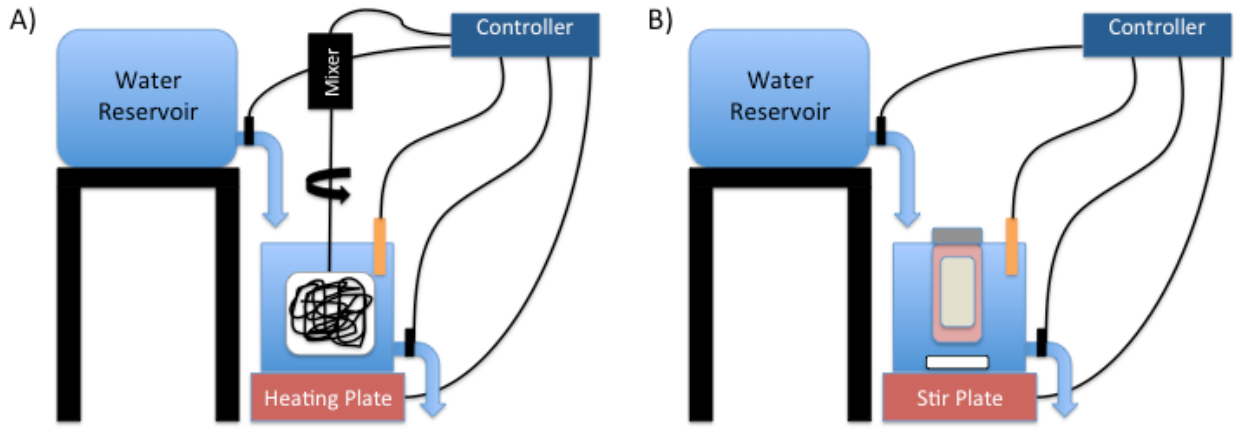


Figure 10. (A) Schematic of silk fiber extraction and (B) silk solution dialysis devices with controlled elements. Controller allows for automated processing of raw silk material. Arrows indicate water influx and efflux, and orange rectangle represents sensors for detecting various processing parameters throughout production.

Significant advances in product sterilization methodology and packaging were undertaken. Although a number of sterilization procedures have been explored previously (i.e. super-critical CO_2 and dry heat) with some success, these previous methods tended to provide either inconsistent results or were not standard sterilization methodologies making future validation protocols somewhat difficult to perform from both a technical and financial standpoint. As a result, the highly consistent and validated E-beam sterilization technique was explored. E-beam sterilization utilizes high-energy electron beams to break the DNA of microorganisms and thus sterilizes the irradiated region. Post-processing analysis of silk samples treated by E-beam with FTIR demonstrated no significant change in protein secondary structure (data not shown), indicating that E-beam does not appear to produce material changes. Silk dissolution studies in water indicated no significant changes between the E-beam treated and untreated controls (Figure 11A), and illustrates this method offers a potential choice for

end-point packaged product sterilization. The packaging design was also completed where the silk bandage product will be placed in a molded plastic base to both assist in handling and protecting the product (Figure 11B). Once the bandage is placed in the plastic molding the combined product and base is placed within a foil pouch, sealed, and sent out for E-beam sterilization.

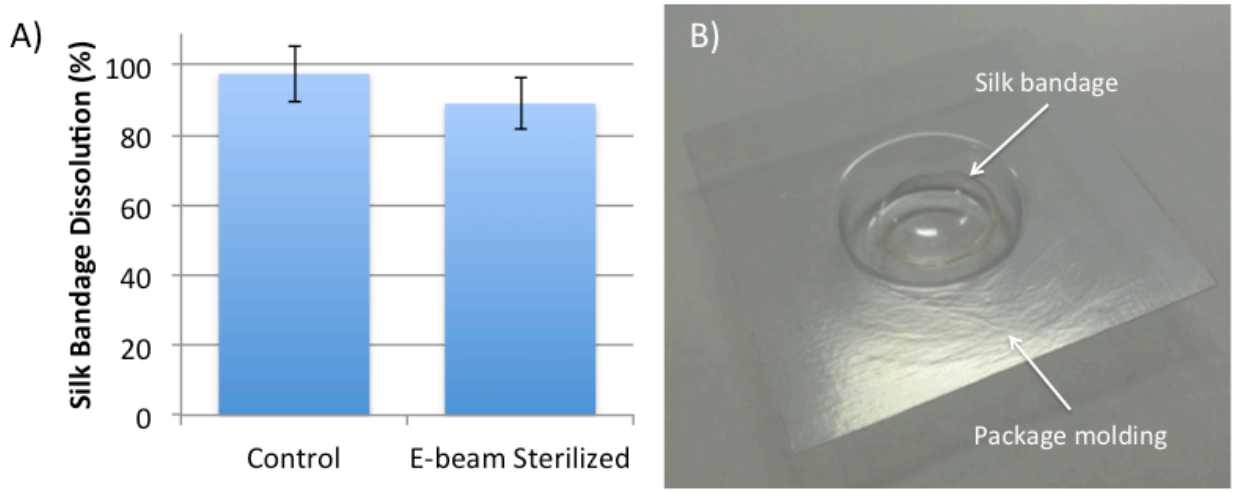


Figure 11. (A) Silk bandage dissolution was verified for samples processed by E-beam sterilization, with no significant differences between processed and unprocessed controls. (B) Formed silk bandage placed within package molding that is then placed in a foil pouch and boxed for E-beam sterilization and shipment.

Problem Areas

(a) Current Problems

The most significant challenge for this project has been in regards to controlling the silk material dissolution properties upon the eye's surface. Although there has been significant progress in understanding this process, there is still further work to be conducted to produce a more consistent and tunable silk dissolution profile that may be optimized for clinical applications. With the addition of the environmental chamber it is anticipated that control over the WA process will be achieved successfully. In addition to technical issues, the project has encountered a number of unanticipated challenges due the company transitioning their technical facilities from the university lab to the manufacturing facility. Currently the company is operating within an ISO 13485 certified facility for medical device manufacturing, which will greatly enhance the speed of the silk bandage product development. As a result of these significant changes, a large

amount of time has been devoted to formulating the transition and implementation plan. However, the company has successfully completed this transition and is at full functional capacity at the new facility.

(b) Anticipated Problems

Due to the continued effort to understand the exact mechanisms underlying the WA process it is anticipated that controlling silk bandage dissolution on the eye surface will continue to provide the greatest technical hurdles to the project. However, now that the project is located to a new facility with greater technical resources it is expected that issues involving silk material dissolution will be better mitigated going forward.

Work to be performed

Task 1. Irritation response

Initial work assessing the silk material's potential as an irritant to the cornea has been completed. Final irritation studies will be undertaken after the final product design has occurred.

Task 2. Burn wound assessment

Initial work assessing the silk bandage's affect on alkali burn wound injuries has been completed. Results indicated that the bandage served well as a potential wound covering for such injuries. Further characterization of the silk bandage's effect on burn wound healing will be assessed after the product design has occurred.

Task 3. Abrasive wound assessment

Initial work assessing the silk bandage's affect on corneal epithelial healing after an abrasive wound has been completed. Results indicated that the bandage served well as a wound covering and also stimulated healing with optimized material residence time on the corneal surface. Further characterization of abrasive wounds will be undertaken after the final product design has occurred.

Task 4. Puncture wound assessment

Before assessing puncture wound applications it was important to understand the potential for material toxicity to the cornea tissue. Initial results indicated that the

potential toxicity of the silk bandage material implanted within the stroma tissue is negligible, if not non-existent, over extended periods of time. With the establishment of this knowledge, further work can progress on developing the silk bandage for puncture wound applications. The next step will be to finalize the silk bandage design, and then apply this device to explanted porcine eye puncture wounds for observation. Pending promising results from these *in vitro* studies an additional *in vivo* follow up study within a live rabbit model may be undertaken. However, work on the puncture wound will be temporarily put on hold until the initial silk bandage design is completed.

Task 5. GMP production of a regenerative lens

In the immediate future a set of residence time studies will be undertaken to assess silk bandage material residence time on the corneal surface using visual inspection through slit lamp monitoring. Results from these studies will indicate the appropriate WA processing parameters required for producing a silk bandage dissolution rate on the cornea surface. This will then be translated to GMP production to make a first product iteration by the completion of the award period. The summary of the GMP development plan in quarterly time periods as follows:

GMP Manufacturing Tasks	2013 Q1	2013 Q2	2013 Q3
Task 1: Silk solution production (Q8-Q9)			
Task 2: Silk bandage processing and manufacturing development (Q9-Q10)			
Task 3: Silk bandage process validation and quality control development (Q10-Q11)			

Administrative Comments

To complete the required animal work a minimum of four trips will be required to the Weill Cornell Medical College by a Sarentis Opthalmics technical representative. These will include two studies to characterize silk film residence time duration, and two studies to characterize the effects of silk bandage application post corneal abrasion. In addition, it is anticipated that a trip will be required for a CDMRP meeting along with an industry

related conference (i.e. ARVO or AdvaMed).

Key Research Accomplishments

- Silk material was determined to be non-toxic within the corneal tissue
- Discovered processing controls to modify silk bandage dissolution
- Successful production of the first silk bandage design iteration
- Attained lab and clean room operational space at a GMP facility
- Addition of technical personal to assist in product development
- Designed required systems for controlling the water-annealing process, silk extraction, and silk solution dialysis
- Determined a variety of sterilization modalities for final silk bandage product
- Completed design and implementation of first silk bandage packaging iteration

Reportable Outcomes

Provide a list of reportable outcomes that have resulted from this research to include:

- Applied for a license from Cornell University for access to claims regarding the corneal bandage product
- PhD attained by Brian Lawrence, now executive and PI at startup company
- Developed rabbit corneal abrasion model for determining the affect of silk materials on and within the ocular surface
- Grant funding in the form of an SBIR phase 2 award was successfully obtained through the NSF in part by the outcomes of this work; a total of $400,000 in private funding was obtained in part by the results of this work through investors

Conclusion

Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

Millions of Americans suffer from painful ocular surface wounds each year. The most severe wounds can cause loss of eyesight. The Sarentis Ophthalmics bandage has the

potential to help heal ocular wounds faster and reduce the risk of vision loss. This regenerative bandage is inexpensive and currently produced in the lab for less than a few pennies. Production is fully scalable to large quantities and can be easily packaged and distributed in a similar fashion as a contact lens. Furthermore, the biomaterial is novel, patented, and new to the medical device industry. It has the potential to be used in conjunction with a majority of ophthalmic procedures.

Work over the course of the past year has been pivotal in translating basic laboratory research to a manufactured medical device. The first year of the award offered data demonstrating the proof of concept for the successful design, fabrication, and animal use of the bandage. The second year built off these foundational discoveries to uncover the mechanism of control over the WA process to enabled controlled material residence time on the cornea. More exciting yet has been the transition of technical work from the university environment to a manufacturing facility, which will enable the production of the bandage for human use over the remainder of the award period. With the successful completion of task 4 and 5 over the next 9 months the corneal bandage is anticipated to be in a position to impact the status of human vision health in a positive direction for the US healthcare system.

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